EXPERIMENTAL STUDIES ON THE MODIFICATION OF SOME OF THE METHODS FOR SPECIFIC ANTI-M, ANTI-N AND ANTI-P SERA PRODUCTION

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The practical need of producing larger quantities of permanently absorbed specific sera is continuously and steadily increasing (6, 9, 16, 18, 20). It is well known that the methods for immunization of animals, mainly rabbits (anti-M and anti-N), and more recently — goats proved to be most useful and successful (anti-P) (7, 8, 10, 12, 14). The forensic medicine practice has extended the use of the specific sera, anti-M and anti-N of the MN system, in solving problems of disputed parentage (18, 20). The credit of having obtained similar sera in small quantities for the first time through immunization of rabbits according to the method of *Race and Senger* (2, 18) goes to Vera Ilieva (1950). Attempts for obtaining anti-P sera before 1965—66 in this country have been never made. The establishing of a serological laboratory at the Chair of Forensic Medicine of the Higher Medical Institute — Sofia made possible experiments on obtaining of specific anti-M and anti-N sera, and later also of anti-P serum which were successfully completed.

However, the rather modest facilities available hardly provided adequate conditions for experimentation on a large number of animals which caused at the very beginning of the project the search of new modifications of the thoroughly studied and already introduced in this country methods of the Central Research Institute — Moscow and of the Forensic Medicine Institute — Humboldt University in Berlin (German Democratic Republic (1, 10, 16, 17).

In order to secure higher efficiency during immunization of rabbits, we embarked on direct intravenous inoculation of 1 cc OMM or ONN 50% suspension of threefold washed out human erythrocytes and sodium chloride $9^0/_{00}$. Preliminary preparation of the animals by means of infusion intraperitoneally, subcutaneously or otherwise was not practiced, and the animals were selected among different breeds — chinchilla, white domestic rabbit, cross-breeds, excluding the wild grey rabbit. Intravenous inoculations were also performed at rather short intervals (every other day) in two 15-day periods, with a 5-day rest between them. After the last injection and before taking the blood samples, we waited for a further period of five days. Crude sera with high titers were obtained allowing for 1:20 or 1:40 dilution after inactivation at 56° C water bath for the absorption. Out of a total of 12 immunized animals, undergoing immunization at such restricted intervals of time with higher percentage erythrocyte suspension,

only three failed to survive the immunization and died during the secondary immunization. The remaining animals produced specific antibodies. This warrants the assumption that the effectiveness of this immunization method is preferable to the prolonged preparation of the animals and particularly, to the immunization of a great number of animals, most of which, although surviving the immunization, fail to produce antibodies in their serum with sufficiently high and practically utilizable titers.

Another difficulty confronted in the obtaining of these sera is their absorption and preservation after absorption. It is well known that Argillas and co-workers, from the Research Institute — Moscow, claim successful production of liquid absorbed anti-M and anti-N sera after numerous successive absorptions, which retained their specifity — preserved in liquid state in ampoules for years (1, 3, 4).

In our experiments, various absorption periods and different amounts of threefold washed out erythrocytes were used: A_1MM and A_1NN. As a rule, the first absorption was started at $^1/_2$ V with one hour absorption period. The following absorptions were never performed during the same or in subsequent days, while preserving the centrifuged and pipette disposed sera in refrigerator at 4° C. The subsequent absorption was performed at intervals of 10—15 days, and in isolated cases, up to the 30th day, but always very attentively and with reduced volume of washed out erythrocytes ($^1/_4$ volume for an hour absorption period). Thereafter, the sera were replaced again in the refrigerator in sterile state at 4° C for 10—15 days and the absorption was repeated with $^1/_8$ volume washed erythrocytes for half an hour. The sera were carefully controlled for specificity and titer. Thus within various absorptional intervals, we obtained permanently absorbed liquid specific sera, which retain their qualities for a long time — in our case, more than 6 months at 4° C. Their preservation at the above conditions is uncertain, since their titer qualities decrease and are lost.

The latter circumstance motivated us to search for and test other preservation conditions for the liquid absorbed sera. It was demonstrated that low temperatures (—20° C) provide favourable conditions for conservation (3. 4, 15). Preserved under such conditions we dispose of liquid absorbed spesific sera, produced in 1962. Nevertheless, after the thawing, their specificity is retained no longer than 5—6 days, if kept in refrigerator at 4° C. In our experience, their preservation at room temperature proved inadequate even in ampoules. We failed to preserve absorbed liquid sera durably at room temperature (3, 4).

For obtaining specific anti-P serum, we resorted to the method of the Berlin Forensic Medicine Institute *Kerde, Fünfhausen and Brunk,* through the immunization of goats (5, 6, 10, 16, 17). Two Kamerun breed goats, aged seven months were immunized. Even at the stage of preparation, we faced serious obstacles in obtaining antigenic liquid from the echinococcus cysts of pork liver. The echinococcus cysts in pigs were a rare occurence and their content was insignificant (5, 6, 8, 11, 12). It may be stated with certainty that in the future, this particular source of obtaining such valuable antigen will decrease all over the world. At the same time, echinococcus cysts in other animals — cattle and sheep — are met with quite frequently, and their bladder content amounts to 500 milliliters (4). The latter circumstance justified the attempt made for obtaining anti-P sera

by means of antigenic liquid, produced from bovine lung echinococcus cysts. The bladder contents were removed in a sterile manner with the addition of 1—2 drops 0,2% solution of boric acid per 50 milliliters echinococcus liquid.

Table I

Goat	Antigenic liquid from echinococcus from bovine lung (intravenous injection of 5 ml)	Sample Blood taking	Blood taking
No. 1	17. XI. 21. XI. 24. XI. 28. XI. 1. XII. 5. XII. 7. XII. 12. XII. 16. XII	17. XII	23. XII
No. 2	17. XI. 21. XI. 24. XI. 28. XI. 1. XII. 5. XII. 7. XII. 12. XII	16. XII	23. XII

We injected 5 cc into the jugular vein as shown in Table I which proved to be of no trouble to the animals. The very first samples prelevated from the animals revealed antibody production in the serum. Blood taking was made on the fifth day after the last injection, without killing the animals. The crude serum obtained after inactivation in water bath at 56° C, for half to one hour, was absorbed consecutively with A_1P-, OP− and BP− threefold washed erythrocytes. With A_2P- absorption was not required. During the process of absorption, it became clear that the initial absorption with A_1P- leads to rapid exhaustion of the serum's specificity with respect to A_2P+ erythrocytes. In this respect the absorption method shown on Table 2 proved to be more suitable.

Table 2

Goats	Time of reaction	With A_1P-	Reference A_1P+	To OP−	Blood OP+	Groups BP−	BP+	A_2P-	A_2P+
No. 1	5 m	—	++	—	±	—	++	—	+
	20 m	—	++	—	+	—	++	—	++
No. 2	5 m	—	±	—	±	—	+	—	±
	20 m	—	+	—	+	—	++	—	+
ABSORPTIONS									
Goat No. 1		1/8 V — 30 m —		1/4 V — 30 m —		1/8 V — 30 m —		—	—
Goat No. 2		1/4 V — 30 m —		1/4 V — 30 m		1/8 V — 30 m 1/8 V 30 m		—	—

As a final result we obtained a specific anti-P serum in both animals with titer 1:64 and 1:128 respectively.

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ЭКСПЕРИМЕНТАЛЬНЫЕ ИЗУЧЕНИЯ МОДИФИЦИРОВАНИЯ НЕКОТОРЫХ ИЗ МЕТОДИК ДЛЯ ДОБЫЧИ СПЕЦИФИЧЕСКИХ АНТИ-М, АНТИ-N И АНТИ-Р СЫВОРОТОК

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РЕЗЮМЕ

Получение специфических анти-М и анти-N сывороток путем иммунизации кроликов, без специальной подготовки животных и их рассы является возможным, при внутривенном вливании 50% суспенсии эритроцитов через сгущенные интервалы. Воз-

можно также получение стойко абсорбированных сывороток путем абсорбции через разреженные интервалы и при внимательном укоречении абсорбционного времени и объемного количества эритроцитов. Стойко абсорбированные сыворотки сохраняются лучше всего при низких температурах, но после размораживания их теряют на несколко дней свою специфичность.

Высокотитрные анти-P сыворотки получают и при иммунизации коз антигенами из эхинококковой жидкости легкого крупного рогатого скота. При ее абсорбировании рекомендуется начальная абсорбция A_1P эритроцитами во избежание снижения их титра.